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Final REPORT ON

CONTRACT NO DA -92-557-FEC-35859

INCLUSIVE DATES 1 March 1962 TO 28 February 1963

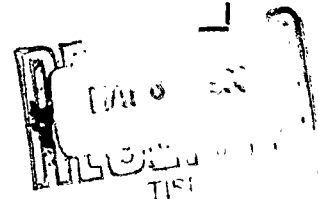
## SUBJECT OF INVESTIGATION

STUDIES ON IMMUNOLOGICAL  
DIAGNOSIS AND THERAPY  
OF PARAGONIMIASIS

## RESPONSIBLE INVESTIGATOR

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MIASIS by Munao Yokogawa.  
Final report No. 1, 1 Mar 62 to 28 Feb 63. 30 p.  
illus. tables, 24 refs.  
(Contract DA92-557-FEC-35859) Unclassified report

In this report the results of the intradermal tests complement fixation test and stool examination which were carried out on more than 11,000 individuals during epidemiological surveys for paragonimiasis during the period from 1954 to 1962 were summarized and analysed.

The results of chemotherapy of paragonimiasis with the various doses of Bitin (Bithionol) and follow-up studies on chest X-ray examination after treatment with Bitin were also reported in this report. (Author)

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2. Epidemiology
3. Diagnosis

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STUDIES ON IMMUNOLOGICAL DIAGNOSIS AND  
THERAPY OF PARAGONIMIASIS.

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### Abstract

#### A. Immunological diagnosis as screening method for paragonimiasis.

A total number of 115,866 individuals were examined intradermal test with V.B.S. Antigen in the endemic and non-endemic areas in Japan during the period from 1954 to 1961. Stool examinations and complement-fixation tests were also performed on those positive and doubtful skin reactors as many as possible.

Paragonimus eggs were all found in the stools of positive skin reactors, but not found in those of negative reactors, accordingly, it was found that the intradermal test was quite specific for paragonimiasis, and was valuable for screening in the large population masses because of simplicity, safety and of low cost. However, positive skin reactors were included both of the present infections and past infections, and were not always shown the present status of lung fluke infections. On the other hand, the complement fixation test was closely correlated with the present infection of lung fluke. Therefore, for the epidemiological surveys for paragonimiasis should be used the intradermal test in the first place, then performed the complement fixation tests and stool examinations on the individuals who showed positive or doubtful skin reactors.

### Abstract

#### B. Chemotherapy of paragonimiasis with Bithionol.

V. Studies on the minimum effective dose of Bithionol and changes of abnormal shadows in chest X-ray examinations after treatment with Bithionol.

80 cases of paragonimiasis were treated with daily dose 20mg/kg - 50mg/kg of Bitin (Bithionol) every other day for 5 - 15 times and completed the follow-up observations for 1 year after the treatments.

As the results, paragonimus eggs in sputum and stools of these patients were disappeared with the administrations from 2 to 5 times of Bitin. 78 out of 80 cases were cured completely. Paragonimus eggs were found again in stools within 3 months after treatment in 2 cases. These 2 relapsed cases were treated with daily dose 40mg/kg of Bitin for 5 times and 20mg/kg for 10 times respectively. Among 129 abnormal shadows found in 78 cases before treatment, 20.9% of them were absorbed immediately after completion of treatment and most of them were absorbed during the period from 1 to 3 months after treatment.

It is consumed that the minimum effective dose of Bitin was daily dose 30mg/kg ~~for 10~~ every other day for 10 times and the chest X-ray examinations after the treatments have practical values to evaluate the efficacies of the treatments for paragonimiasis.

A. IMMUNOLOGICAL DIAGNOSIS AS THE SCREENING METHOD  
FOR PARAGONIMIASIS IN THE ENDEMIC AREA  
OF PARAGONIMIASIS.

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## Introduction

Recent studies indicate that the prevalence and the public health significance of paragonimiasis in the Far East, especially in Japan, Korea and China is greater than previously estimated. It has been written in the text book that paragonimiasis can be diagnosed clinically by a persistent cough and the character of the sputum. An unequivocal diagnosis of this infection depends on the recovery of Paragonimus eggs from the sputum or stools. The recovery of eggs, however, is not easy and often very difficult in cases which are mild, latent or therapeutically affected. Until recently most of the surveys for paragonimiasis in an endemic area of paragonimiasis have been made also by recovery of eggs in sputum or stools. Much time and expenses can be saved by the easy and simple method of diagnosis for epidemiological survey for paragonimiasis.

A considerable amount of works on immunological diagnosis has been done especially in the last few years with intradermal test. Since the screening method for paragonimiasis by means of intradermal test has been rather carefully standardized in the preparation of antigen and its use by one of the authors (M. Yokogawa, 1955), the intradermal tests have been widely used in Japan in surveys to determine the incidence of lung fluke infections. A few attempts have also been made to use the complement fixation tests as aids for the laboratory diagnosis of paragonimiasis. (Ando, 1921. Yokogawa, M. et al. 1956. Chung et al. 1956. Sadun et al. 1958). The authors reported that the complement fixation test can be used as a criterion of the effectiveness of the treatments for paragonimiasis. (Yokogawa, M. 1962) This paper reports the practical values of the intradermal test and complement fixation test as the screening methods for paragonimiasis.

## Materials and Methods

The authors and co-workers have made the surveys for paragonimiasis in the last 8 years from 1954 to 1961 in which over 110,000 persons were given intradermal test. In these surveys as many as possible of those positive reactions to the intradermal tests were given from two to three stool examinations. The complement fixation tests were also performed on those positive reactions to the intradermal test from 1958 to 1961. These surveys were made in six endemic areas and 4 non-endemic areas of paragonimiasis in Japan. (Fig. 1)

### 1) Stool Examinations

From two to three examinations were performed on the individuals who gave positive skin reactions. The AMS III concentration technique (Hunter et al. 1948) was used.

## 2) Intradermal test

The antigen (V.B.S. antigen) from adults of Paragonimus westermani was used in the test. Its preparation has been reported in detail by M. Yokogawa, (1955).

For the intradermal test 0.01 ml. of the antigen diluted to 1:10,000 with 0.01 % merthiolate in normal saline is injected intradermally into the left forearm: this is enough to raise a wheal of 3 to 4 mm in diameter. To read this test the wheals is measured immediately after injection and again after 15 minutes. The reaction usually appears one to two minutes after injection, reaches its maximum within 15 minutes and disappears within 3 to 6 hours. The wheal is usually accompanied by redness and in many cases pseudopodia are present. Any reaction is considered positive in which the diameter of the wheal (average of length and width of the wheal) exceeds by 5 mm the diameter of the wheal raised immediately after injection; Reaction in which the diameter exceeds by 4 mm is considered doubtful and by 3 mm or less negative. A control injection is not necessary.

## 3) Complement fixation test

The complement fixation test was conducted in the manner described by Kent and Mayer (1948). The technique employing the 50 percent end point titration of complements and the utilization of two and half 50 per cent hemolysis units were proved to be superior and more sensitive by the authors. (Yokogawa, M. et al. 1955).

1 to 5,000 of V.B.S. antigen prepared from adults of Paragonimus westermani as the same method of V.B.S. antigen for intradermal test. More than 1 to 10 in dilution of the antiserum at the 50 per cent hemolysis and point was considered positive. The serum taken from the person who showed positive or doubtful skin reactions was inactivated at 56°C for 30 minutes as soon as sero-preparations and kept in -20°C. The serum was inactivated again at 56°C for 10 minutes before the test.

## Results and Discussion

A total of 115,866 individuals were tested in 6 endemic areas and 4 nonendemic areas of paragonimiasis in Japan from 1954 to 1961. (Fig. 1) Although positive dermal reactors were found in every area examined, the percentage varied greatly between the endemic areas and non-endemic areas as shown in Table 1.

Less one per cent of the individuals examined was positive in non-endemic areas and no Paragonimus eggs were found in those of positive skin reactions. Paragonimus eggs were found in those of positive skin reactions in every endemic area in some degree, however, recovery rate of paragonimus

eggs from positive skin reactors varied from one endemic area to another. It can be seen from the Table that the percentage of positive for Paragonimus eggs is the highest in Niigata area in spite of the low percentage of positive skin reactions. What differs from other endemic areas is the fact that the individuals examined in Niigata area are all school children from 10 to 18 years old.

Table 2 shows the results of the intradermal test on school children only in two other areas. It can be seen from the Table that the percentages of positive for Paragonimus eggs in these three areas are also high compared with those of general population including school children shown in Table 1.

Detailed studies were carried out in the whole population of Tsushima-machi, Ehime prefecture to investigate the relationship between the results of the intradermal test and actual infections. The whole population (95.6 % of a total population) was given intradermal test, complement fixation tests and from 2 to 3 stool examinations simultaneously.

This locality has been known to be high endemic area of paragonimiasis in Japan. A relatively high number of dermal reactors was found in this locality. The number of persons tested and the number of positive dermal reactors are shown in Table 3 according to age and sex. The number of individuals who showed Paragonimus eggs in stools was also shown in the same Table. Paragonimus eggs were found in stools from 30 out of the 180 positive dermal reactors and 1 out of 71 doubtful dermal reactors. Of a total of 31 individuals who showed Paragonimus eggs, 22(39.2 %) were found from the 56 positive dermal reactors between 10 and 19 years of age.

One of the authors, Yokogawa, M. (1956) reported that four individuals who had recovered from paragonimiasis and their sputa and stools had been negative for eggs for from 20 to 30 years and the complement fixation tests were negative, still showed positive skin reactions. This fact may suggest that the positive skin reactions to V.B.S. antigen continue for long period, more than 20 years after the complete recovery from paragonimiasis.

In Table 4, the results of the Complement fixation test performed on the same individuals in Tsushima-machi mentioned above. The percentage of positive complement fixation tests varied greatly in each age group. These individuals who showed positive complement fixation tests were all positive for the intradermal tests.

In male 15 out of 30 individuals who gave positive Complement fixation tests were found in the age group of 10 to 19 and 13 out of 15 individuals belonging to this age group were positive for Paragonimus eggs in stools. In female 13 out of 28 individuals were also found in this age group and 9 out of 13 showed Paragonimus eggs. In both of male and female the number

of the positive reactions in the complement fixation test and the number of those who showed paragonimus eggs were significantly greater in this age group 10 to 19 than those in any other age group. Two individuals who showed positive skin reactions and Paragonimus eggs, gave negative reactions in the complement fixation test in females.

It seems to be evident that the results of the complement fixation test is well correlated with active infections of paragonimiasis. However, it should be noticed that a few cases which showed positive intradermal test and paragonimus eggs, gave negative reactions in the complement fixation test.

In the surveys since 1958 the complement fixation tests were performed as many as possible of those who showed positive or doubtful skin reactions. These results are shown in table 5 according to the areas surveyed. In the endemic areas a total of 3,477 individuals who showed positive or doubtful skin reactions were given complement fixation tests. Of a total of 3,477, 1,063 or 30.6 % were positive reactors in the complement fixation test in the endemic areas. On the other hand, of 209 positive skin reactors in the non endemic areas. Only 12 or 5.7 % were positive in the complement fixation test and no Paragonimus eggs were found from those of positive reactors in the complement fixation tests. Of a total 1,063 individuals of those positive for the complement fixation tests in the endemic areas, 428 or 40.1 % were positive for paragonimus eggs and 3 individuals showed paragonimus eggs inspite of negative reactions in the complement fixation test. Any significant difference was not found in the percentages of positive reactors in the complement fixation tests from positive skin reactors among these six endemic areas.

It can be safely said that the results of the complement fixation test is closely correlated with the active infections. On the other hand the percentage of positive for eggs from those of positive complement fixation test varied greatly among these areas. These results have been pooled for the analysis of this fact. Relation between the antibody titers in the complement fixation test and the egg positive cases are shown in Table 6. It can be seen from the Table that the number of positive for eggs increased with the increase of the complement fixation titer in the group which showed positive skin reactions. 91 of 129 cases which showed more than 10 to 160 antibody titers, were positive for eggs in stools. It is not yet known exactly about this reason. From our experience the antibody titers in the complement fixation test has no relation with the number of parasites, but has rather close relation with the activity of the worm parasitized. Any significant difference was found in the percentages of positive complement fixation tests between males and females.

It has been said that the intradermal injection of antigen may itself provoke antibodies. The authors investigated consecutively the changes of

intradermal tests after turning negative for the Paragonimus eggs by the treatment with Bithionol which was found to be quite effective by the authors (1961, 1962). Besides the authors attempted to observe the effect of the intradermal test to the partial immunity of sensitization of the skin by the repeated inoculations of antigens at the same location of the same individuals. The intradermal test with V.B.S. antigen and complement fixation test with V.B.S. antigen were performed on 16 patients and 2 healthy individuals as control. These tests were conducted before the treatment, immediately after the completion of the treatment and every month after the treatment for 1 year. As shown in Table 7 the tendency to reduce or increase the diameters of the wheals with this antigen was not found. The complement fixation tests of all the patients treated with Bithionol became negative during the period from 1 month to 5 months after treatment and no influences of the repeated injections of the antigen to the provocation of the antibody of the complement fixation test were seen.

From the above mentioned results it seems to be clarified the practical values of the intradermal test and Complement fixation test as the methods of the epidemiological surveys for paragonimiasis. The intradermal tests with V.B.S. antigen can be used to great advantage for screening large population masses because of its simplicity, safety, ease of application and low cost. However, it can be said that the results of the intradermal tests does not always show the present status of lung fluke infections in the area examined because of the continuation of the positive dermal reaction for long period after recovery of the disease. On the other hand, the complement fixation test is closely correlated with the present status of infection and not past infections. Therefore, for the epidemiological surveys the intradermal test should be used in the first place and the complement fixation tests should be performed on the individuals who showed positive or doubtful dermal reactions.

Cross reactions of complement fixation test to other diseases and purified antigens for these tests were also investigated, (Yokogawa, M. et al. 1958, 1959), but these results were not discussed here.

#### Summary

The authors and co-workers have made the surveys for paragonimiasis in Japan in the last 8 years from 1954 to 1961 in which 115,866 persons were given intradermal test. In these surveys as many as possible cases of positive skin reactors were given from two to three stool examinations. The complement fixation tests were also performed on those positive skin reactors from 1958 to 1961. Each practical value of the intradermal test and complement fixation test as the screening methods for paragonimiasis was highly evaluated from these results.



It was found that the intradermal test was quite specific for paragonimiasis and all of those who showed Paragonimus eggs in stool were found from those of positive skin reactors and no Paragonimus eggs were found from those of negative skin reactions.

However, in those of positive skin reactions both of the present infections and past infections were included. On the other hand, the complement fixation test showed positive reactions only in those of active infection. As previously mentioned, it seemed to be advisable in the survey for paragonimiasis the intradermal test should be given at first to all persons of the area and the complement fixation test and from 2 to 3 stool examination should be given those of positive for the intradermal tests. Consequently the results of the complement fixation test of the area surveyed may indicate directly the incidence of infections of paragonimiasis.

It may be advisable that school children would be suited for the subject of the survey to determine the incidence of present lung fluke infections in the proper area.

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Table 1. Results of intradermal tests using V.B.S. Antigen in the endemic areas of paragonimiasis and the non endemic areas in Japan. (1954 to 1961)

(M. Yokogawa and co-workers)

Areas	Skin Test		Stool Examination	
	No. exam.	No. posit. (%)	No. exam. (posit. or doubtful by skin test)	No. posit. for eggs (%)
Tohoku (General population) school children	3,663	93(2.5%)	67	153 12( 7.8%)
Niigata (school children)	16,950	382(2.3%)	91	312 108(34.6%)
Noto (General population) school children	6,046	168(2.7%)	78	236 32(13.5%)
Shizuoka (General population) school children	26,374	848(3.1%)	163	832 76(10.5%)
Ehime (General population) school children	15,170	1,485(9.8%)	447	1,221 153(12.5%)
Kochi (General population) school children	29,065	1,073(3.6%)	750	723 47( 6.5%)
Total	97,268	4,049(4.2%)	1,596(1.7%)	3,477 428(12.3%)
Hokkaido (school children)	4,768	14(0.3%)	5	19 0(0.0 %)
Toyama (school children)	2,319	29(1.3%)	34	62 0(0.0 %)
Kaga (school children)	6,387	51(0.8%)	59	77 0(0.0 %)
Fukui (school children)	5,124	25(0.5%)	35	49 0(0.0 %)
Total	18,598	119(0.6%)	133(0.7%)	209 0(0.0 %)
TOTAL	115,866	4,168(3.6%)	1,729(1.5%)	3,686 428( 1.6%)

Table 2. Results of Intradermal test for paragonimiasis  
on school children.

Areas surveyed	Skin Test		Stool Examination	
	No. examined	No. positive and percent	No. examined (Posit. or doubt. for skin test)	No. Posit. for Eggs (%)
NIIGATA	16,950	382 (2.3%)	312	108 (34.6%)
SHIZUOKA	4,351	155 (3.5%)	133	57 (42.8%)
EHIME	3,269	156 (4.7%)	156	65 (41.6%)
Total	24,570	693 (2.8%)	601	230 (38.3%)

Table 3. Results of Intradermal tests according to Age and Sex.

Age group	Number examined		Positive reaction			Doubtful reaction			
	Male	Female	Both	Male	Female	Both	Male	Female	Both
1-9	111	96	208	13(10.6%) (1)	4( 4.0%)	17( 8.2%) (1)	4	1	5
10-19	131	117	248	30(22.8%) (13)	26(22.3%) (10)	56(22.5%) (23)	11	3	14
20-29	58	58	116	25(43.2%) (1)	14(24.1%) (2)	39(33.6%) (3)	5	2	7
30-39	67	77	144	29(43.3%)	19(24.7%)	48(33.3%)	6	6	12
40-49	46	77	123	21(45.6%)	23(29.9%)	44(35.7%)	4	3	7
50-	86	120	206	43(56.0%) (2)	23(19.3%) (1)	66(32.0%) (3)	12	14	26
Total	500	545	1,045	161(32.2%)	109(20.0%)	270(24.8%)	42(8.4%)	29(5.3%)	71(6.8%)

. . . . . Number in parenthesis shows number of individuals who showed Paragonimus eggs in stools.

Table 4. Results of Complement fixation tests according to Age and Sex.

Age group	Male	Female	Both	Complement fixation test			
				Positive reaction		Negative	
				Male	Female	Both	Male
0-9	112	96	208	3( 2.6%) (1)*	4( 4.2%)	7( 3.4%) (1)	109
10-19	131	117	248	15(11.4%) (23)	13(11.1%) (9)	28(11.3%) (22)	116
20-29	58	58	116	4( 6.8%) (1)	6(10.3%) (1)	10( 8.6%) (2)	54
30-39	67	77	144	0	1( 1.2%)	1( 0.7%)	67
40-49	46	77	123	1( 2.1%)	0	1( 0.8%)	45
50-	86	120	206	7( 8.1%) (2)	4( 3.3%) (1)	11( 5.3%) (3)	79
Total	500	545	1,043	30( 6.0%) (17)	28( 5.1%) (11)	58( 5.5%) (28)	470
							512 (2)
							987

\* ..... Number in parenthesis shows number of individuals who showed Paragonimus eggs in stools.



Table 5. Result of Complement fixation test performed on the individuals who showed positive or doubtful skin reactions in the surveys for paragonimiasis from 1958 to 1961.

Areas	No. exam. by Skin test	*No. exam. by C.F. test and stool exam.	No. Posit for C.F. test	(%)	No. Posit. for Paragonimus eggs	(%)**
Tohoku	3.663	153	34	(21.8%)	12	(35.3%)
Niigata	16.950	312	153	(49.0%)	108	(70.5%)
					(1)***	
Noto	6.046	236	88	(37.2%)	32	(36.3%)
Shizuoka	26.374	832	241	(29.0%)	76	(31.1%)
Etchime	15.170	1.221	334	(27.2%)	153	(46.0%)
					(2)***	
Kochi	29.065	723	213	(29.5%)	47	(22.0%)
Total	97.628	3.477	1.063	(30.6%)	428	(40.1%)
					(3)***	
Hokkaido	4.768	19	3	(15.7%)	0	0.0
Toyama	2.319	64	2	( 3.0%)	0	0.0
Kaga	6.387	77	5	( 6.5%)	0	0.0
Fukui	5.124	49	2	( 4.0%)	0	0.0
Total	18.598	209	12	( 5.7%)	0	0.0

\* Positive or doubtful skin reactors.

\*\* No. posit. for Eggs/No. posit. for C.F. test. \*\*\* No. posit. for Eggs from negative C.F. test.

Table 6. Comparison of the Results of Complement Fixation Tests, Intradermal Test and Stool Examinations according to the Antibody Titers of Complement Fixation Tests.

Sex	Result of Skin Test	No. exam. by C.F. Test	No. Posit. for C.F. Test	x10-x20	x21-x40	x41-x80	x81-x160	x160 over
Male	doubtful reactions	194	33(17.0%) (6)*	11 (5)	5	11	4 (1)	2
	positive reactions	1.115	454(40.7%) (232)	136 (41)	86 (41)	91 (52)	65 (43)	76 (55)
Female	doubtful reactions	162	29(17.9%) (7)	11 (4)	8 (1)	5	4 (1)	1 (1)
	positive reactions	722	284(39.3%) (121)	78 (24)	82 (24)	37 (17)	34 (19)	53 (36)
Total	doubtful reactions	356	62(17.4%) (13)	22 (9)	13 (1)	16	8 (2)	3 (1)
	positive reactions	1.837	738(40.2%) (353)	214 (65)	168 (65)	128 (70)	99 (62)	129 (91)

\*..... Number in parenthesis shows Number of individuals who showed paragonimus eggs in stools.

Table 7. Changes of the increase of diameters of Wheals with VBS Antigen on the Individuals before, during and after Treatment with Bithionol. (unit; mm.)

Case No.	Age	Sex	During Treat.										Av.	
			Before Treat.	10th	20th	1	2	3	4	5	6	9		12
1	12	M.	4	5	4	7		5	5	6		3	5	5.5
2	14	F.	13	11	10	8		13	9	9			10	10.3
3	14	M.	14	10	8		10	9	9	9	10		12	10.1
4	14	F.	11	11	10	7	7	10	7	7		8	8	8.5
5	42	M.	14	8	12	12	15	22		15	9		14	13.4
6	18	M.	8	14	8	8								9.5
7	32	M.	8	7	8	7	10	8	9	8	8	9	16	8.9
8	59	M.	18	9	11	13	7	14	16	16	7		14	12.5
9	62	F.	17	18	16	8	17	10	16			14	11	14.1
10	16	M.	17	18	5	5	8	6	9	8			9	9.4
11	49	F.	8	6	8	11	8	10	16	7	9	7	11	9.2
12	38	F.	13	10	8	8	6	9	7		9	11	6	8.6
13	65	M.	6	5	9	8	8	8	7			7		7.3
14	31	M.	12	15	17	10	22	14	18	13		12	16	14.9
15	20	M.	11	8	8		15							10.5
16	59	M.	11	10	9	8	18	8	11	10	9	10	9	10.3
Average			11.6	10.3	9.4	8.6	11.6	10.1	11.0	9.8	8.5	9.0	10.8	10.2
(Control)														
1	9	M.	3	3	1	1	1	0	0	0	0		0	0.9
2	13	M.	1	3	0	0	0	1	0	0	0		0	0.5
Average			2.0	3.0	0.5	0.5	0.5	0.5	0	0	0		0	0.5

B. CHEMOTHERAPY OF PARAGONIMIASIS WITH BITHIONOL. V. STUDIES ON  
THE MINIMUM EFFECTIVE DOSE OF BITHIONOL AND CHANGES OF  
ABNORMAL SHADOWS IN CHEST X-RAY EXAMINATIONS  
AFTER TREATMENT WITH BITHIONOL.

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## Introduction

The large size of the worms and their position within cysts in the lung tissue have added to the difficulties in finding a satisfactory treatment for paragonimiasis. Until now a large number of different drugs were tried, none of which showed any promise except emetine hydrochloride and chloroquine phosphate. However, even the combined method of treatment with emetine hydrochloride and sulfonamids, a method most prevailing, gives a recurrence rate of 70 to 80 % after treatment of only one course for 10 to 14 days. It has been also reported that the use of chloroquine phosphate for such a long term as from 93 to 231 days shows a remarkable effect, but such continuous administration as requiring a long term like this has been proved not to be applicable in Japan because of its side-effects. And yet the effect can hardly be expected with administration of chloroquine phosphate for two or three weeks.

Yokogawa, M. et al. (1961, 1962 a, b, c,) reported that paragonimiasis can be easily cured completely with Bithionol (Bitin ; Tanabe Co. Japan) given in the daily dose of 50 mg/kg every other day for 5 to 15 doses and no relapses were observed for one year after treatment with this drug. Yokogawa, M. et al. (1962) also reported that Bitin can be used for mass treatment of paragonimiasis in the endemic area as the control measure of paragonimiasis.

The present investigation was undertaken to secure additional informations on a relation between the amount of Bitin used and its efficacy, as well as on the changes taking place in the abnormal shadows in chest X-ray findings after treatment with Bithionol.

## Material and Method

Bithionol is a tasteless, odorless and white crystalline powder, having a chemical structure as shown in Fig. 1, showing skin sterilizing effect, and has been used under the commercial name, Actamer (Monsanto Co.) the United States as an ingredient of soap or cosmetics. In Japan this agent was found, for the first time to have an excellent anti-helminthic effect on the chicken tapeworm Raillietina Kashiwarensis and liver flukes of cattle, Fasciola hepatica (Sawada, 1957, Ueno, et al. 1959). Yokogawa M. et al (1962) also used Bithionol successfully for Taenia saginata and Diphyllbothrium latum of human type worms. This drug is now put out by Tanabe Co. Japan as Bitin for human use. In this trial Bitin was used for treatment of paragonimiasis.

### 1) Objects

All the patients who have been diagnosed as paragonimiasis at National Kochi Hospital, Japan, during the period from August, 1960 to March, 1962, have been treated with Bithionol, its number having reached 110 at present.

Most of these patients have ever been treated with combined method of Emetine Hydrochloride and sulfonamide, but since this treatment was found fruitless, they came this hospital looking for the new treatment with Bithionol. Follow-up observations were made on 62 adults and 18 children, 80 cases in total out of those treated with Bitin for more than 6 months after the completion of treatment.

## 2) Amount and Method of Administration

In this study both inpatients and outpatients were treated with Bithionol. On the inpatients various examinations were carried out every day and on the outpatients were made every two or three days.

Yokogawa, M. et al (1962) have reported that from the follow-up observations for one year after the treatment of 13 cases consisting of 8 cases of 15 administrations 4 cases of 10 administrations and one case of 5 administrations, each taking 50 mg/kg of Bithionol per day immediately after each meal on alternate days, complete recoveries were observed in whole cases, and its minimum effective dose was left still needing further inquiry. Thereupon the amount of Bithionol to be applied was tentatively made 50 mg/kg, 40 mg/kg, 30 mg/kg and 20 mg/kg and assorted into four groups, and their effects were compared.

As seen in Table 1, the number of cases in each group by dose of Bithionol was as follows: per day 50 mg/kg administration 23 cases, 40 mg/kg 27 cases, 30 mg/kg 17 cases and 20 mg/kg 13 cases. The way of administration was, as a rule, oral administration of one third of the daily dose immediately after each meal every other day and the number of takes was 10 or 15 times, but in a few cases, there were also 5 or 7 administrations.

## 3) Method of Examinations

As for inpatients, during the period of administration of Bithionol, the whole sputa were collected every day and after examination of their properties and amounts, they were fully dissolved with 2 % NaOH, solution and centrifuged. The total number of eggs in these sediments was counted. For stool examination

AMS III Method (0.5g of stool used) was applied and the number of eggs per 1 gm of feces (EPG) was calculated. As for outpatients, sputa and feces on every day were also brought forward and egg counting was made in a similar way. After the completion of treatment stools and sputum were examined on consecutive 3 days every month. The decision of complete cure was given to the case in which no eggs of Paragonimus were found in both of sputa and feces for more than 6 months. In addition bloods were taken before treatment and every month after treatment and the complement fixation test for paragonimiasis was carried out and the change in the antibody titers in this test due to the treatment was investigated. Moreover chest X-ray examinations with both of ordinary (direct) radiography

and tomography were made before and every month after treatment, and the state of absorption or contraction of abnormal shadows in the lungs was pursued periodically.

#### Results of Treatment

As indicated in Table 1, perfect cure was observed in 18 cases of 15 times administrations and 1 case of 5 times administrations with 50 mg/kg per day. In the group administered with 40 mg/kg per day, perfect cure was observed in 14 cases of 15 times administrations and 12 cases of 10 times administrations, but after 7 and a half months Paragonimus eggs were found again in one case of 5 times administrations. In the group administered with 30 mg/kg per day, complete cure was observed in 2 cases of 15 times administrations, 14 cases of 10 times administrations and one case of 7 times administration and in the group administered with 20 mg/kg per day it was observed in 4 cases of 15 times administrations but in the third month from the completion of treatment, bloody sputa and Paragonimus eggs were again found in one case out of 9 cases of 10 times administrations. Namely it comes to the fact that in the group administered with from 30 mg/kg to 50 mg/kg per day, all of 64 cases given more than 10 times administrations every other day were regarded as perfect cure. Since one case out of nine cases given 10 times administrations with 20 mg/kg per day showed a recurrence, the amount meeting the expectation for sure, perfect recovery, that is, the minimum effective dose of this agent is thought to be 10 times administrations of 30 mg/kg every other day. Moreover one case of 5 administrations of a daily dose of 50 mg/kg every other day did not show any recurrence even one and half a year after treatment, and this can be looked upon as perfect cure. But in one case administered 5 times with a daily dose of 40 mg/kg every other day, bloody sputa were found after 7 and half a month, in which Paragonimus eggs were again detected. Whether this is due to recurrence or reinfection is not yet obvious, and further investigation will be made into the cases of five times administrations hereafter.

- 1) Times of Disappearance of Bloody sputa and Eggs during the treatment with the various amount of Bithionol

The frequency of bloody sputa and the existence of eggs in the bloody sputa were examined on consecutive days after the onset of the internal use of Bithionol with 41 cases who complained bitterly of bloody sputa before treatment, these bloody sputa always containing eggs. In the group of in patients, the disappearance of bloody sputa and eggs in them was observed after the second administration in 7 cases out of 14 cases hospitalized and in other 7 cases after from the third to 7th administrations, as shown in Table 2. The disappearance of bloody sputa was also observed after the second administrations in 10 cases, 3-5 times administrations in 15 cases, and 7 and 8 times administration in 2 cases, out of 27 out patients. That is, the

disappearance of bloody sputa and eggs in them were noted mostly in the oral administration of this agent less than 5 times with both groups of in- or outpatients. The time of disappearance showed no remarkable difference according to the daily dose from 30 mg/kg to 50 mg/kg. In addition, the relation between the frequency of bloody sputa and the number of times of the administrations of the drug per day was examined on 3 cases showing bloody sputa with particularly high frequency out of these 41 cases as shown in Fig. 2. Namely, although the case "u" showed bloody sputa on almost consecutive days for about 6 years until this treatment and also 17 times bloody sputa per day were noted before administration, 4 times oral administrations, namely 8 days after the initiation of treatment, caused perfect disappearance of bloody sputa. The case "t" who had excreted bloody sputa, 13 times per day before treatment, is one of examples needing comparatively a large number of days. Even in this instance, however, bloody sputa disappeared on the 16th day, that is after 8 times administrations.

Paragonimus eggs found in the bloody sputa after the 5th administration were all deformed or degenerated and the sputum was light yellow and mucous rather than bloody.

## 2) Changes in Eosinophilic cells

Although eosinophilic cells in the blood of paragonimiasis patients do not necessarily show an remarkable increase, it is generally said that abnormally high percentages are occasionally shown at a time immediately after infection or in the heterotopic parasitism of Paragonimus, for a instance, in subcutaneous paragonimiasis. 13 cases mentioned in Table 3 were all examples of chronic patients and before treatment showed an increase in eosinophilic cells by 6-16 % (8.0 % average) but the month after the completion of treatment the percentage of eosinophilic cells showed 0-5 % (2.3 % average) and there was merely one case that showed as high as 5 % in the highest. It is interesting that the percentage of eosinophilic cells became normal soon after the complete recovery from paragonimiasis with Bithionol.

## 3) Side effects

Diarrhea, loose stools, abdominal pain, nausea, vomiting or urticarial eruption has been mentioned as the side effects of Bithionol by Yokogawa M. et al (1961). In the present study, too, on the occasion of internal use of Bithionol, although diarrheal stools were initially noted once to several times per day, stools became in part naturally to the normal during the continual administration of the drug, and there was none that discontinued the intake of the drug because of diarrhea or that needed a symptomatic treatment. Diarrhea was noted in 32 out of 62 adult cases (51.6%) and in 3 out of 18 children cases (16.7 %) both being transient. In addition, abdominal pain, anorexia, nausea or vomiting was encountered, but were all slight. Moreover,



urticarial eruption or dermatitis were observed in 6 cases of adults and 1 case of children, totally 7 cases, as noteworthy side-effects. A transient albuminuria was proved in 2 cases showing urticarial eruption, but no abnormality was found in the function test for liver and kidney and the result of electrocardiogram was also normal. When the relation between the amount of Bithionol administered and the frequency of side effects is observed, it may be said that smaller the amount of Bithionol administered is, the lower the rate of appearance of side effects becomes. Meanwhile when adults and children were compared, the rate of appearance of side effects in children was obviously lower.

4) Change of the abnormal shadows in chest X-ray pictures after treatment with Bithionol.

The chest X-ray pictures of paragonimiasis are roughly classified into 1) infiltrative shadow, 2) ring shadow or nodular shadow, (cystic shadow) 3) bronchiectasia, 4) increased lung markings and 5) no change and among them infiltrative shadow and ring shadow or nodular shadow have been said to be comparatively characteristic to this disease. In addition, tunnel-like shadows, (Iwasaki, 1955), are occasionally encountered and rarely enlargement of hilus or calcification shadows are observed. The course how the chest X-ray shadows in paragonimiasis change with the death of worms due to the Bithionol treatment is thought to be a highly interesting problem. Accordingly a periodical pursuit of X-ray shadows were attempted with 78 cases which had been regarded as having cured perfectly by the internal use of Bithionol. The course of infiltrative, ring or nodular shadows which had been noted prior to treatment were examined by taking normal and tomographic X-ray pictures immediately after the treatment and every month thereafter until the 6th month. As shown in Table 4, 129 shadows were observed in 78 cases before treatment, but immediately after completion of Bithionol treatment 27 shadows out of 129 shadows (20.9%) had disappeared by absorption, and those in which the contraction or the reduction of shadows were noted came to 83 (64.3%) and those without any change were merely 19 (14.7%). In the total number from one to three months after the treatment those disappeared were 79 (61.2%), those of contraction or reduction were 41 (31.8%); from 4 to 6 months after treatment, disappearance 98 (76.0%), contraction or reduction 26 (20.2%); and those showing no change in shadows even after 6 months were only 1.5 (3.9%), which produced entirely no change even after one year. The relation between the property of the shadow and the time of its disappearance, contraction, or reduction was investigated, revealing no definite relation. In general, however, it seems that uniform infiltrative shadows regarded as comparatively fresh ones disappear most quickly, ring shadows or nodular shadows are readily absorbed in this order, and uneven infiltrative shadows are latest in absorption. As for the change of shadows such as absorption, reduction or contraction, there was no difference depending upon the amount of Bithionol administered within the range of daily dose from 20 mg/kg to 50 mg/kg. In the following four cases will be mentioned so as to illustrate

the change of X-ray shadows due to the treatment with Bithionol.

#### Case A

K. S. Age; 21, male, a company employe, Tosa, Kochi prefecture.

Braunish sputa were excreted once-twice per month since April, 1956, visited a certain hospital in Osaka under diagnosis as pulmonary tuberculosis, and despite a chemotherapy for tuberculosis for about a year, sputa did not disappear and no change occurred in the chest X-ray shadows. In July 1960, eggs of Paragonimus were initially found in sputa and about 20 injections of Emetin hydrochloride were applied, but the disappearance of sputa as well as eggs were not noted. Hospitalized in our hospital, Kochi National Hospital on February 2, 1961. According to the view at the time of admission, the constitution was the average large and the body weight was 51.8 kg. The intradermal reaction for paragonimiasis was positive, tuberculin reaction positive, blood sedimentation value 2 mm (one hour), in sputa tubercule bacillus was negative with smear test and culture test, and eggs of Paragonimus were found. In stools eggs of Paragonimus were also found, Ocult blood test negative, normal in the urine test, hemoglobin index; 96 %, the number of erythrocytes was  $447 \times 10^4$ , the number of leucocyte 9,400, Eosinophile cell 9 % and complement fixation test for paragonimiasis was positive. (Antibody titers X 80)

Treatment was initiated on February 3, 1961 and 2.0g of Bithionol per day (40 mg/kg) was applied 15 times after each meal on every other day until March 2. Bloody sputa disappeared after the second administration of Bithionol (on the fourth day from the start of oral administration), and Paragonimus eggs found in the stool were all deformed on the 5th day. No Paragonimus eggs were found in stools, and sputa as well became disappeared on the 6th day. (after third administration of Bithionol) In the chest X-ray picture before treatment, diffused infiltrative shadow was observed in the middle of the right lung as seen in Plate 1 (A<sub>1</sub>). But this shadow completely disappeared immediately after treatment as seen in Plate 1 (A<sub>2</sub>). Eosinophilic cells became 1 %, complement fixation test for paragonimiasis a remarkable reduction of an antibody titer from X 80 to X 14 was observed 1 month after treatment, and he left the hospital on March 3. Complement fixation reaction turned to negative at the test in April (2 month after treatment), and judging from the results of stool examinations for eggs, no relapse was noticed until February, 1962. (13 month after treatment) It may be added that there was only a tendency to produce loose stools for two or three days after the start of use of the drug as a side-effect of Bithionol.

#### Case B

N. M., Age: 12 years, male, a shoolchild, Inomachi, Takaoka-gun, Kōchi.

On the occasion of physical-examination held in 1954 (first year of a primary school) an abnormal shadow was found in the chest X-ray picture, received a diagnosis as pulmonary tuberculosis and treated with SM and PAS for 3 months, but no change occurred in the abnormal shadow found in the lungs

before treatment. On 11, Feb. 1955, Paragonimus eggs were detected in his stools at our hospital and 4 courses of treatment with Emetine hydrochloride and sulfonamide was given repeatedly but no effect was found. (on course of treatment: 14 days) Every year abnormal shadow in the lungs was pointed out at the physical-examination, occasionally when taking cold or after violent motions bloody sputa continued, but in these two years this seemed to have stepped.

On Jan. 6, 1961 came to this hospital again. The growth was good showing a body weight of 41 kg. X-ray picture showed a nodular shadow in the middle lobe of the left lung as seen in Plate 1 (B<sub>1</sub>) which manifested little difference from that before 6 years. In addition, intradermal reaction for paragonimiasis was positive, tuberculin test was positive, blood sedimentation value; 2 mm, (one hour) hemoglobin index; 98 %, erythrocyte count, 503 X 10<sup>4</sup>, leucocyte count; 7,200, eosinophile cells; 6 %, eggs of paragonimus were noticed in stools but other kinds of helminths eggs were not found, and urine test was normal. The complement fixation test for paragonimiasis was strong positive (antibody titer: X 118).

Treatment by visiting hospital was begun from Jan. 7, 1961, 1.5g of Bithionol (about 40 mg/kg) per day was given orally after breakfast and dinner by halves, totally 10 times every other day until Jan. 25. Entirely no side effect was noticed. In the X-ray picture taken on the 10th day from the completion of treatment, namely about one month after the beginning of treatment, as seen in Plate 1 (B<sub>2</sub>), nodular shadow as noticed before treatment was fairly absorbed. Eosinophilic cells decreased from 9 % to 2 %, antibody titer in the complement fixation test decreased from X 118 to X 32. In the fifth month from the completion of treatment, as seen in Plate 2 (B<sub>2</sub>), the shadow was perfectly absorbed, and the complement fixation reaction became negative, Eggs of Paragonimus were still negative for more than 1 year after treatment.

#### Case C

H. U., Age; 8 years, male; a school child, Kōchi-shi.

In August, 1956 suffered from measles and was complicated with exsudative pleurisy of right side. After the improvement of pleurisy, abnormal shadows were left in the right lung, and no change of shadow was found even after the chemotherapy with SM and PAS for 3 months. In Oct. 1959, examined at tuberculosis Preventing Association in Kochi city, tuberculin test was negative, intradermal test for paragonimiasis was positive, eggs of Paragonimus were found in stools; 7 courses of treatment of Emetine hydrochloride and sulfonamide was given repeatedly for a year until the end of the 1960 (one course of treatment; 14 days), but the disappearance of abnormal shadows in chest X-ray picture were not observed.

On Feb. 6, 1961, visited the hospital. The constitution was slightly small, the body weigh 20 kg, subjective symptoms such as cough, sputum, or others was not complained. The chest X-ray picture before the treatment with

Bithionol, ring shadows were seen in the middle of the right lung as shown in Plate 2 (C<sub>1</sub>). Tuberculin test was negative, intradermal reaction for paragonimiasis was positive, blood sedimentation titer was 7 mm at one hour, hemoglobin index; 69 %, erythrocyte count;  $352 \times 10^4$ , leucocyte count; 6,800, eosinophilic cells; 6 %, eggs of Paragonimus were found in stools, and the complement fixation test for paragonimiasis was strong positive. (antibody titer  $> \times 160$ )

A daily dose of 0.8g of Bithionol (40 mg/kg) was administered every other day, divided, after breakfast and supper, from Feb. 7, to Mar. 7, for 15 doses. As the side-effects, a little reduction of appetite was observed but diarrhea was not, and after the 13th administration of Bithionol small papules appeared scattering symmetrically on both cheeks but disappeared soon after the completion of administration. The course of disappearance of the shadows in chest X-ray picture was as follows which can be seen in photographs: immediately after completion of the administration, ring shadows became obvious on the contrary as compared with those before treatment, as shown in Plate 2 (C<sub>2</sub>), but after one month ring shadows together with infiltrative shadows around them were fairly absorbed as seen in Plate 2 (C<sub>3</sub>), and in the tomogram after 5 months, as shown in Plate 3 (C<sub>4</sub>), these shadows had perfectly absorbed. Eosinophilic cells came to 0 % immediately after the completion of administration and the complement fixation test for paragonimiasis turned negative after 5 months. More than one year has elapsed already with this case, but no appearance of relapse has been caught.

#### Case D.

Y. M., Age 17 years, male, a pupil of middle school, K<sup>-</sup>ochi.

In spring 1954 bloody sputa were expectorated, abnormal shadows were found in the right lung by X-ray examination subjected to the chemotherapy for pulmonary tuberculosis but these shadows remained unchanged and bloody sputa as well were not removed, and thereupon visited this hospital to receive examination. As the result intradermal reaction for paragonimiasis was positive, tuberculin test was weak positive, eggs of Paragonimus were detected in the sputa, but tuberculous bacillus was not found in both smear as well as culture test. 3 courses of treatments with Emetine hydrochloride and sulfonamide were given without making no effect, and blood sputa were still maintained after violent movements.

On March 22, 1962, infiltrative shadows were observed in the lower of the right lung by X-ray examination as seen in Plate 3 (D<sub>1</sub>), entirely in the same manner as that at the time of initial examination before 8 years. Paragonimus eggs were found in sputa as well as in stools, eosinophilic cells were 8 % and the body weight was 45 kg.

From March 23 on a daily dose 1.8g (40 mg/kg) of Bithionol was administered

after each meal being divided into 3 takes every other day, and on March 28, the day next to the day when the third administration of Bithionol had finished, urticarial eruption was produced over the whole body. The infiltrative shadow in the X-ray picture taken on this day increased with the urticarial eruption as seen in Plate 3 (D<sub>2</sub>) but by continuing the administration of Bithionol along with the symptomatic treatment for urticaria, ten times administrations of Bithionol was concluded without any other abnormality. In Plate 3 (D<sub>3</sub>) taken on April 13 immediately after the completion of administration, the abnormal shadow were perfectly absorbed. Eosinophilic cells were also reduced to 2 %.

#### Discussion

As stated above, a daily dose of Bithionol was classified into 4 groups 50 mg/kg, 40 mg/kg, 30 mg/kg, and 20 mg/kg, and a treatment for paragonimiasis with these daily doses was attempted. As the result, all those cases administered with more than 30 mg/kg daily dose from 10 to 15 times every other day showed a perfect cure. With 20 mg/kg 4 cases administered 15 times every other day showed complete cure without exception but of 9 cases administered 10 times one case appeared to have a relapse.

Sato et al (1962) reported that they observed a perfect cure even in cases administered with a daily dose of 10 mg/kg once a week, if the number of administrations was over 10 times. Katamine et al (1961) also reported that with the amount of administration with a daily dose of 20 mg/kg or 30 mg/kg of Bithionol every other day or even every three days, Paragonimus eggs could be eliminated in a short period of time. Accordingly, it seems necessary to study further on the amount of administration as well as on the interval of administration of Bithionol but viewed from the author's result mentioned above, administrations of a daily dose of from 30 mg/kg to 40 mg/kg every other day, more than 10 times, may be said as the safest and surest method at present. Daily doses grouped against ages may be tentatively mentioned as follows.

Age	Daily dose of Bithionol
over 13 years	1.5 - 2.0 g
9 - 12 years	1.0 - 1.2 g
5 - 8 years	0.6 - 0.8 g
below 4 years	0.2 - 0.4 g

As regards the side-effects of Bithionol, Sato, Katamine and others stated that diarrhea, nausea, vomiting, abdominal pain and so on were observed but they were not so serious that the administration should be stopped, and also in the author's observations, there was no cases in which marked side-effects were observed. However, seeing from the relation between the frequency of occurrence of side-effects and daily dose, a tendency that the

less the daily dose, the less the occurrence of side-effects was noted.

Some consideration will be given on the time of recurrence of one case administered 5 times with 40 mg/kg which seemed to have relapsed after treatment with Bithionol together with one case out of those administered 10 times with 20 mg/kg. The former case was a sailor and therefore exact pursuit of the time of relapse after treatment was not necessarily possible. As he noticed the bloody sputa seven and half months after treatment, he visited our hospital and Paragonimus eggs were found again in bloody sputa and stools. As for the latter case, bloody sputa were initially observed and Paragonimus eggs were detected in the examination made on the 4th month after the treatment. Yokogawa has informed that the absence of Paragonimus eggs for more than 3 months after the treatment may be regarded as a perfect cure, deducing from the fact that in cases of relapse after the treatment with Emetine hydrochloride and sulfonamide, Paragonimus eggs were again found almost within one month after the completion of treatment. Basing upon this view, it seems a little difficult to look upon above two cases for recurrence. because the detection of Paragonimus eggs after treatment take place after more than 3 months from the conversion to negative for Paragonimus eggs. Yokogawa et al, however, lately experienced such cases as follows,

A Korean, male, 37 years old, who had been suffered from stubborn bloody sputa for 7-8 years, received treatments for paragonimiasis at several hospitals in Tokyo, revealing no effect. Learning by chance the talk of Bithionol visited the author's laboratory. In his feces Paragonimus eggs and clonorchis sinensis eggs were detected. The treatment with a daily dose of 50 mg/kg of Bithionol for 15 doses was initiated on August 6, 1961. As the result, bloody sputa disappeared on the 8th day from the start of administration (after 4 times administrations) and Paragonimus eggs became negative in the examination of feces. In the examination of feces in the 4th month therefrom, Paragonimus eggs were not found and not only bloody sputa but also mucous sputa were not excreted at all, being in a very good condition. However, Clonorchis sinensis eggs were still found in his feces. The antibody titer in the complement fixation test, which showed more than X 160 before the treatment, rapidly reduced following to X 13. 4 months, after treatment (December, 1962) but it did not reach a negative value. After then, examination could not be made owing to his own circumstances. Meanwhile the examination of feces for other purpose was held by a certain laboratory, Tokyo, in March, 1962, and it is said that a small number of unknown helminth eggs having remarkably deformed were detected in addition to the eggs of Clonorchis sinensis. As for the origin of these unknown eggs no special attention has been paid. On June 5, this patient visited the laboratory wishing for the treatment for clonorchiasis, and on examining feces a small number of helminth eggs of little small shape, having indistinct opeculum and irregular form, and slightly yellow colored, were found. The examination of eggs was further repeated but normal eggs of Paragonimus were not found at

all. He had no subjective symptoms such as bloody sputa and others and abnormal shadows looking like a focus can not be observed either in the X-ray plane photography or tomography, but as the result of repeated examination of feces, a diagnosis for these eggs was given that they are deformed Paragonimus eggs. Besides, in the complement fixation test an antibody titer still showed X 11 showing weakly positive. After then Bithionol was administered again with a daily dose of 50 mg/kg every other day for 10 times and after the third administration this deformed eggs had already disappeared and on this occasion the complement fixation test three months after the second treatment with Bithionol showed a perfect conversion to negative. On the basis of this fact, it may be thought rather reasonable to construe that above case is not a reinfection, but the parasite which had been injured by the former treatment recovered vitality. Such revival of Paragonimus eggs as appearing after a long term from the treatment has not been encountered in the treatment with Emetine hydrochloride, and it is necessary to inquire whether the worms injured once could recover vitality to produce again normal eggs or not and also to study further on the standard for the criterion of complete cure. Now, as often pointed out by Yokogawa, the point of conversion to negative in the complement fixation test is thought to have an important significance in the decision of cure of this disease.

In the next place, the shadows in the X-ray pictures of the lungs suffered from paragonimiasis was pursued periodically after treatment and it was found that the period for its disappearance is unexpectedly short. This may be construed to be due to the quickness of the restoration of lung tissues with the death of Paragonimus worms in the worm cyst.

Although a definite relation was not necessarily found between the property of shadows and the time of its disappearance as well as contraction, it seems that generally speaking, uniform infiltrative shadow is the promptest in absorption, followed by ring or nodular shadows, and uneven infiltrative shadow appears latest. It is thought to be the most interesting problem to inquire into the relation between these shadows and practical symptoms in the lung through pathological anatomy.

On the other hand, in one case during the treatment with Bithionol (on the 5th day from the start of the treatment, that is, after 3 times administrations) and in the other one case in the X-ray photograph immediately after the completion of treatment, though limited only in above two cases, there appeared a transient increase of shadows of focus. The reason for this has not been clarified, it may be supposed that this may be based on the allergic reaction the host body due to the sudden death of parasites, and as for this point further investigation is desirable to be made in the future.

### Summary

Since the early August, 1960, the authors treated 80 patients of paragonimiasis with internal use of Bithionol, a daily dose of from 20 mg/kg to 50 mg/kg, and followed up the course after the completion of administration during from a half to one year, obtaining the following result.

#### 1. Method of administration of Bithionol:

A daily dose of from 30 mg/kg to 40 mg/kg, orally administered 10 times every other day, suffices a satisfactory effect and for children, considering the point of less side-effects, the amount commonly used should be as follows; 1.5-2.0g for higher than 13 years, 1.0g for 9-12 years, 0.6g for 5-8 years, 0.2g for lower than 4 years.

Out of these 80 cases, those having shown relapses until now are limited in only two cases, one in the cases administered 10 times with 20 mg/kg and one in the cases administered 5 times with 40 mg/kg, and with these cases administered more than 10 times with more than a daily dose of 30 mg/kg, there have been found no recurrence. In one case administered 5 times with 50 mg/kg a perfect cure was observed, but further inquiry should be required for the reduction of number of times in administration from this. As side-effects are not so remarkable even with administrations on alternative days carried out by the authors, compulsive extension of the term of treatment is thought unnecessary.

2. Most of Paragonimus eggs in the bloody sputa or feces of patients suffering from paragonimiasis disappeared by from 2 to 5 times administration of Bithionol and those who showed an increase in eosinophilic cells turned normal a comparatively short time after the completion of treatment.

3. Diarrhea, abdominal pain, nausea, vomiting and ruticarial eruption or dermatitis were observed as the side-effects of Bithionol, but were transient. Especially in case of children, the frequency of occurrence of side-effects were markedly lower than that of adults.

4. On fifth of chest X-ray shadows of paragonimiasis was absorbed immediately after the treatment with Bithionol, most of shadows were absorbed after from one to three months, and those remaining unchanged for longer than 6 months up to one year, were only a few. In consequence it is thought possible to decide the effect of treatment comparatively early by pursuing the change of X-ray shadows of lungs after treatment.

5. It was found that Bithionol was not effective for clonorchiasis though limited in only one case.

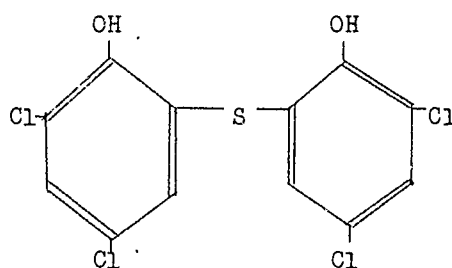


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Fig. 1. Chemical structure of Bithionol



2,2'-thiobis (4,6 dichlorophenol)

Table 1. Result of treatment with Bithionol for paragonimiasis

Daily dose	Method of administration	No. of doses administered	No. of cases treated	No. of cases cured*	No. of cases recurred
50mg/kg	internal use every other day	15	18	18	0
	"	10	4	4	0
	"	5	1	1	0
40mg/kg	internal use every other day	15	14	14	0
	"	10	12	12	0
	"	5	1	1	1
30mg/kg	internal use every other day	15	2	2	0
	"	10	14	14	0
	"	5	1	1	0
20mg/kg	internal use every other day	15	4	4	0
	"	10	9	8	1
Total			80	78	2

\* follow-up more than 6 months after treatment.

Table 2. Relation between No. of doses administered and disappearance of bloody sputum

No. of doses	Inpatients No.* of cases	Outpatients No.* of cases	Total
1	1	2	3
2	6	8	14
3	3	7	10
4	3	5	8
5	1	3	4
6	0	0	0
7	0	1	1
8	0	1	1
9	0	0	0
10	0	0	0
Total	14	27	41

\* No. of cases in which bloody sputum disappeared with administration of Bithionol.

Table 3. Changes of eosinophilic cells before and after treatment with Bithionol.

Cases	Before treatment Eosinophilic cells (%)	Immediately after treatment Eosinophilic cells (%)
M.R.	9	0
H.K.	6	3
M.K.	6	1
T.M.	7	3
N.Y.	15	4
M.M.	8	3
I.S.	16	4
U.G.	5	2
A.S.	6	1
N.M.	13	5
M.Y.	6	2
Y.K.	13	4
U.K.	6	0
Average	8.9	2.5

Fig. 2. Times of disappearance of bloody sputum and Paragonimus eggs

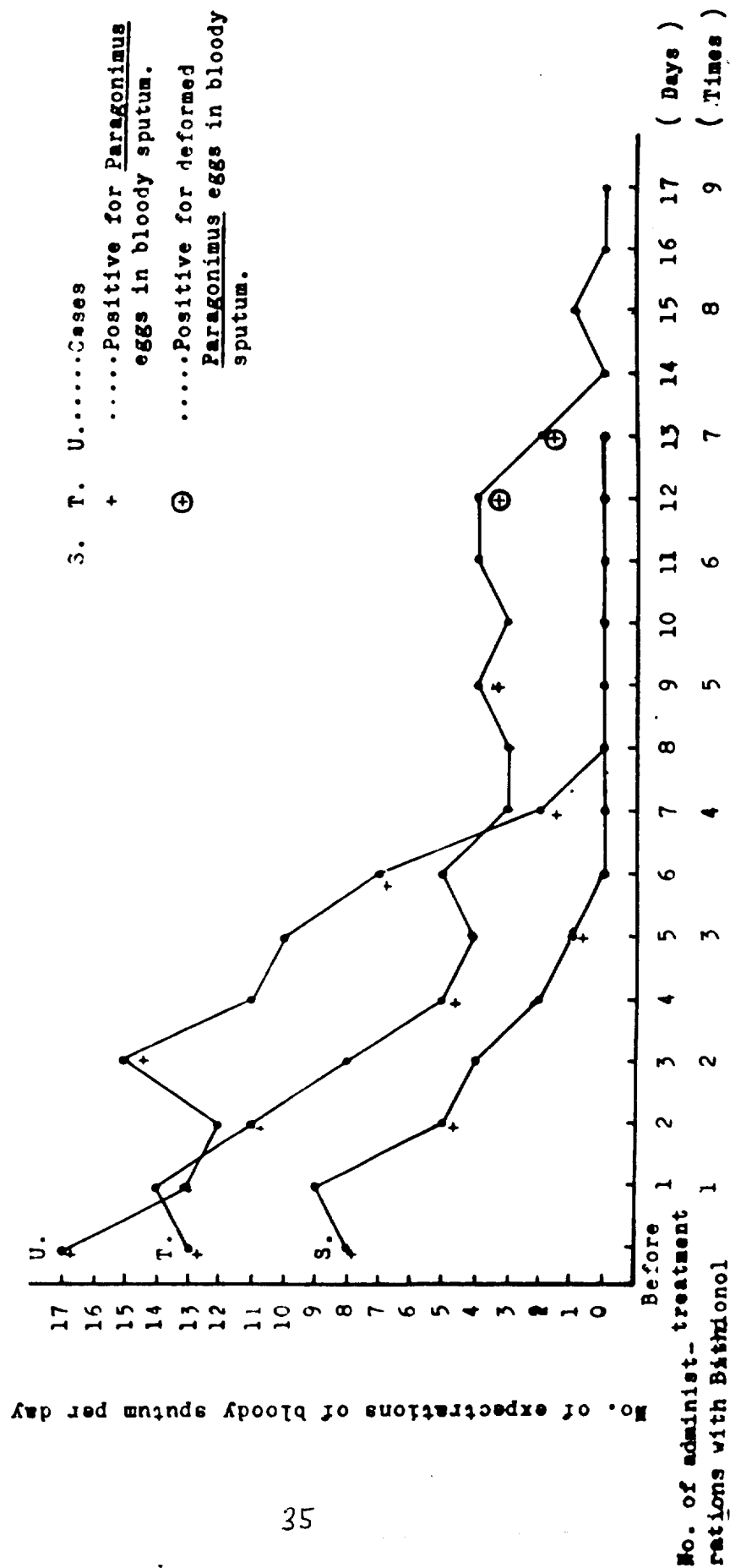


Table 4. Changes of abnormal shadows in Chest X-Ray examinations after treatment.

(129 pathological shadows in 78 cases)

Lapse of time after treatment	No. of abnormal shadow			Total
	Disappeared	Reduced	Unchanged	
Immediately after treatment	27 (20.9%)	83 (64.3%)	19 (14.7%)	129
1 - 3 months after treatment	78 (61.2%)	41 (31.8%)	9 ( 7.0%)	129
4 - 6 months after treatment	98 (76.0%)	26 (20.2%)	5 ( 3.9%)	129



Plate

- A1. Case A: Before treatment, diffused infiltrative shadow was seen in the middle of the right lung.
- A2. Case A: Immediately after treatment, diffused infiltrative shadow disappeared completely.
- B1. Case B: Before treatment, the nodular shadow was seen in the middle of the left lung.
- B2. Case B: 10 days after treatment, the shadow was fairly absorbed.
- B3. Case B: 5 months after treatment, the shadow was perfectly absorbed.
- C1. Case C: Tomogram before treatment, the ring shadows were seen in the middle of the right lung.
- C2. Case C: Immediately after treatment, the ring shadows and the infiltrative shadow around them were seen obviously.
- C3. Case C: 1 month after the completion of treatment, both of the shadows were fairly absorbed.
- C4. Case C: 5 months after treatment, the shadows were perfectly absorbed.
- D1. Case D: Before treatment, infiltrative shadow was seen in the lower of the right lung.
- D2. Case D: Immediately after the 3rd administration of Bithionol, the infiltrative shadow increased with the urticarial eruption.
- D3. Case D: Immediately after the completion of treatment, the shadow was perfectly absorbed.



A1



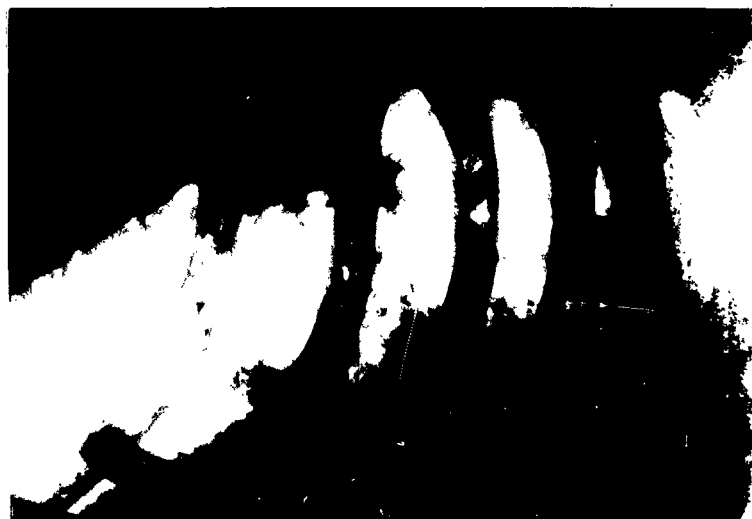
A2



B1



B2



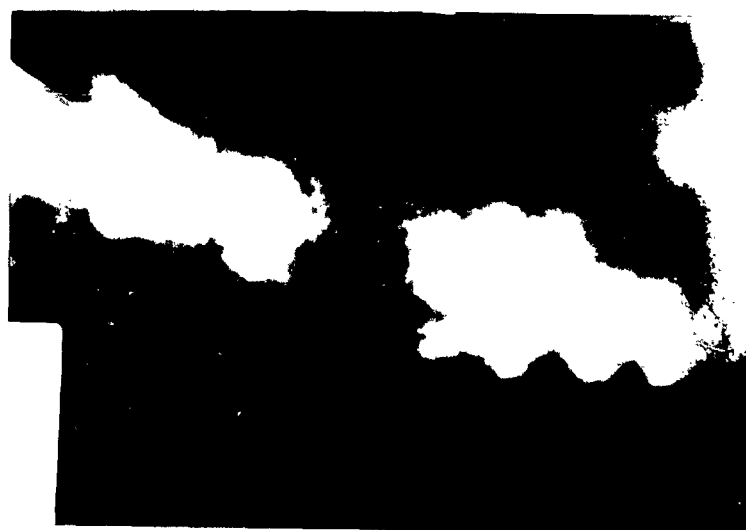
53



54



02



03



D1



D2

D2



D3



(1)

(2)

(3)

(4)